In the Specification

Replace the section "Cross Reference to Related Applications" with the following:

Cross-Reference to Related Applications

This application is a divisional application of 10/223,160, filed August 19, 2002, issued as U.S. Patent No. 6,680,426, which is a continuation of 08/972,901, filed November 18, 1997, now abandoned; which is a continuation of 08/278,555 filed July 20, 1994, issued as U.S. Patent No. 5,693,507; which is a continuation of 07/985,451, filed December 3, 1992, now abandoned; which is a continuation of 07/638,565, filed January 7, 1991, now abandoned; which is a continuation in part of 07/249,616, filed September 26, 1988, now abandoned; to all of which the benefit of priority is claimed under 35 USC § 120. ~~This application is a continuation in part of U.S. Patent Application Serial No. 249,616 filed September 26, 1988 which disclosure is hereby incorporated by reference.~~

Replace the paragraph beginning on page 3, line 8 with the following:

Relevant Literature

Uptake and expression of bacterial and cyanobacterial genes by isolated cucumber etioplasts (immature chloroplasts) has been described. Daniell and McFadden, Proc. Nat'l Acad. Sci. (USA) (1987) 84: 6349-6353. Stable transformation of chloroplasts of Chlamydomonas reinhardtii (a green alga) using bombardment of recipient cells with high-velocity tungsten microprojectiles coated with foreign DNA has been described. See, for example, Boynton, et al., Science (1988) 240: 1534-1538; Blowers, et al. Plant Cell (1989) 1: 123-132 and Debuchy et al., EMBO J. (1989) 8: 2803-2809. The transformation technique, using tungsten microprojectiles, is described by Klein ~~Kline~~ et al., Nature (London) (1987) 327:70-73. Manipulation of chloroplast genes has been described, for example, generation of chloroplast mutants, Maliga et al., Nature (1975) 255: 401-402; protoplast fusion, Belliard et al., Mol. Gen. Genet (1978) 165:231-237; organelle inactivation, Avid et al.., Plant Cell Rep. (1986) 3: 227-230; and chloroplast recombination, Medgyesy et al., Proc. Nat'l Acad. Sci. USA (1985) 82: 6960-6964.

Replace the paragraph beginning on page 5 line 3 with the following:

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the plasmid pHD407 which carries a 4.1-kbp *Sma* I fragment insertion containing the origin ~~orrigin~~ of replication (D loop) from pea cpDNA inserted into pHD312. The plasmid pHD312 contains the entire promoter and 5' untranslated region of the pea *psb*A gene inserted 5' proximal to the promoterless *cat* gene present in the promoter selection vector pkk232-8.

Replace the paragraph beginning on line 15, page 6 with the following:

Figure 5 shows kinetics of chloramphenicol acetylation with [^{14}C] acetyl CoA, in tobacco NT1 protoplasts electroproated with foreign DNA. Pelleted protoplasts were resuspended in (400 µl) extraction buffer (5 mM EDTA/0.25M Tris-HCL, pH 7.8, and 1.0 µg each anrtipain and pepstatic per ml), sonicated for 20 sec, and centrifuged in a Microfuge for 5 min at 4°C to pellet the debris. The extract (190 µl) after heat treatment (65°C, 10 min) was mixed with [^{14} C] acetyl CoA (0.1 µCi; 1 Ci = 37 GBq) and chloramphenicol (40 µl of 8 mM stock). The reaction was carried out at 37°C using the highly quantitative two-phase assay system described by Neumann ~~neumann~~ et al. (26). Slope values derived from data points and DNA concentrations used for electroporation ~~eletroporation~~ were as follows: pUC19, 31.0 (15 µg); pHD407, 24.2 (50 µg); 35S-CAT, 114.1 (15 µg). The correlation coefficient varied between 0.9 and 0.99 for different sets of experiments.

Replace the paragraph beginning on line 23, page 7 with the following:

Figure 8 shows expression of pHD203-GUS over time. Cells collected on filter paper by vacuum filtration were transferred onto NT1 medium solidified with 0.2% gelrite ~~geirite~~ and bombarded with helium entrainment configuration at 1500 psi, sample 8.1 cm from launch point using 1.0 sleeve level. At each time point (days after

bombardment), cells were assayed for GUS activity. In both experiments, each treatment contained 3 replicates.

Replace the paragraph beginning on line 35, page 7 and bridging to page 8 with the following:

Figure 10 shows effect of osmoticum on chloroplast transformation. NT1 cells collected on filter paper by vacuum filtration were transferred onto 0.2% gelrite ~~geirite~~ solidified NT1 media supplemented with different concentrations of osmoticum (1/2 sorbitol and 1.2 mannitol), incubated for at least 1.5 hours and bombarded. After three days of incubation, the cells were assayed for GUS activity. In experiment 1, each treatment had 5 replicates, and in experiment 2, each treatment had 4 replicates.

On page 8, replace the paragraph beginning on line 8 with the following:

BRIEF DESCRIPTION OF THE SPECIFIC EMBODIMENTS

Methods and compositions are provided for obtaining cells containing chloroplasts into which heterologous DNA has been inserted. The method includes the steps of preparing an expression cassette. By expression cassette is intended a DNA construct comprising a coding sequence and appropriate control sequences to provide for proper expression of the coding sequence in the chloroplast. The expression cassette generally includes the following minimum components, the 5' untranslated region from a microorganism gene or chloroplast gene such as psbA which will provide for transcription and translation of a DNA sequence encoding a polypeptide of interest in the chloroplast; a DNA sequence encoding a polypeptide of interest such as genes which provide for herbicide resistance or encode insecticidal proteins; and a translational and transcriptional termination region such as a 3'; ~~2'~~ inverted repeat region of a chloroplast gene that could stabilize RNA of introduced genes, thereby enhancing foreign gene expression. A host cell which contains chloroplasts is transformed with the expression cassette and then the resulting cell containing the transformed chloroplasts is grown to express the polypeptide of interest. The cassette may optionally include an antibiotic resistance gene in addition to a mutated native chloroplast gene such as rbcL or

16SrRNA. In this option, expression of a desirable alternation of a native protein may be favored in transformed chloroplasts by antibiotic selective pressure.

On page 10, replace the paragraph beginning on line 25 and bridging to page 11, line 12, with the following:

For transcription and translatin of the DNA sequence encoding a polypeptide of interest, the entire promoter region from a gene capable of expression in the chloroplast general is used. The promoter region may include promoters obtainable from chloroplast genes, such as the psbA gene from spinach or pea, the rbcL and atpB promoter region from maize and rRNA promoters. Examples of promoters are described in Hanley-Bowdoin and Chua, TIBS (1987) 12:67-70; Mullet et al., Plant Molec Biol. (1985) 4: 39-54; Hanley-Bowdoin (1986) PhD. Dissertation, the Rockefeller University; Krebbers et al. , Nucleic Acids Res. (1982) 10: 4985-5002; Zurawaki et al., Nucleic Acids Res. (1981) 9:3251-3270; and Zurawski et al., Proc. Nat'l Acad Sci. U.S.A. (1982) 79: 7699-7703. Other promoters may be identified and the relative strength of promoters so identified ~~idenntified~~ evaluated, by placing a promoter of interest 5' to a promoterless marker gene and observing its effectiveness relative to transcription obtained from, for example, the promoter from the psbA gene, the strongest chloroplast promoter identified to date. The efficiency of foreign gene expression additionally may be enhanced by a variety of techniques. These include the use of multiple promoters inserted in tandem 5' to the DNA sequence of interest, for example a double psbA promoter, the addition of enhancer sequences and the like.

On page 15, replace the paragraph beginning at line 27 with the following:

A plant containing transgenic chloroplasts may be generated when the host cell used in the transformation process possesses totipotency. Procedures for regeneration of transgenic plants from transformed cells or tissues are, in general, similar, with suitable modifications within the capability of one skilled in the art. Regeneration of dic such as sugar beets, Freytag et al. Plant Cell Rep. (1988) 7:30-34; tobacco, Svab et al. Proc. Nat'l Acad. Sci. U.S.A. (1990) 8526-8530 or monocots such as wheat from anthers or embryos (see below) routinely has been successful.